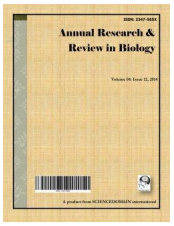

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Disentanglement of *Staphylococcus aureus* in Chicken by Enzyme-Linked Fluorescent Assay

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

This study is aimed to detect *Staphylococcus aureus* (*S. aureus*) in the chicken using conventional methods (biochemical tests), latex agglutination, and Vitek Immuno Diagnostic Assay System (VIDAS) system. These methods are considered as the modern methods to proven technology for the detection of foodborne pathogens. In this study, 100 samples were collected from the supermarkets in Baghdad city. The isolated colonies were picked up by Chromogenic and Baird Parker Medium and transferred onto a fresh medium to ensure purity based on phenotypic characteristics, biochemical tests, AIP® Staph kit, and the detection of enterotoxin using VIDAS SETII. The results were showed that all samples were decontaminated by *S. aureus* and toxic. Toxin A, B, and C has high-frequency of 0.25-2.00 ng g^{-1}, 1.00 ng g^{-1}, and 0.25 ng g^{-1}, respectively. The toxin (A) was found to be more than (10^4 – 10^5) CFU ml^{-1}.

Keywords: S. aureus; enterotoxin; AIP® Staph kit; VIDAS SETII enterotoxin kit.

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1. INTRODUCTION

Staphylococcus (Gram-positive cocci) has arranged in cluster. It was the facultative anaerobe and catalase positive, but *S. aureus* and *S. saccharolytic* were anaerobic and coagulase-negative, respetively [1]. *Staphylococcus* (Seven species) were known as coagulase-positive or variable reactions (*S. aureus, S. intermedius, S. schleiferi, S. hyicus, S. lutrate, S. delphini, and S. pseudintermedius*) [2]. The coagulation production was correlated with the pathogenicity of these bacteria and the *Staphylococcus* (CNA) were minor pathogens [3]. *S. aureus* is one of the important bacterial pathogens in soft tissue infection, UTI, and other diseases [4,5]. The effect of *S. aureus* in animals, mastitis, wound infection, arthritis, and other diseases was found [6]. It was found in the food to be 27.4% in Brazil, was isolated from some food and meat in Turkey, and was found in fascist food [7-9]. Ses and Set were known (SE-like) and syndrome toxic 1 (t st^{-1}) [10]. Enterotoxin of *S. aureus* R (Ser) was similar to toxin [11]. Studies were indicating that the gene responsible for enteric toxin was by toxin (A), so most cases of *staphylococcal* belong to this toxin [12]. In this study, modern technology for the detection of foodborne pathogens was used, which is depended on VIDAS SET11 system. The VIDAS SET 11 has specificity and sensitivity (TRANSIA plat) for a measure of enterotoxin of *S. aureus* [13]. It has a good quality and sensitivity compared with other techniques in microbiology of the food [14]. A comparison between VIDAS and other methods for measurement of toxins were conducted [15]. This study was aimed to identify the *Staphylococcus aureus* in the chicken meat and measurement of the toxin using VIDIS SET II, which is considered more sensitivity to *S. aureus* toxins.

2. MATERIALS AND METHODS

One hundred chicken breast samples were collected from different supermarkets in Baghdad city, Iraq. Potassium Tellurite (P.T) solution was prepared by adding 100 ml of distilled water to 2.8 g (P.T). Baird-Parker Media was prepared by adding 1000 ml of distilled water to 63.5 g of media and was sterilized using an autoclave. Then, egg yolk of 50 ml was mixed with P.T of about 6 ml. Chromogenic Media (C.M) was prepared by adding 100 ml of distilled water to 3 g of C.M and was sterilized using the water bath at 100°C (10 min). Only this media was not sterilized using an autoclave because the chromogenic substrate is very sensitive to heat, which is lead to damage or denaturation. The Staph API system was consisted the plastic tape included 20 tubes, each one has a small amount of agar media, that is useful in identifying a characteristic of *S. aureus*, as shown in Table 1. Table 1 includes the type of test, the symbol, how to infer the negative, and positive results either directly or after the addition of the detectors. This system was used according to the instructions of the company with the following steps:

1. Prepare a suspension with a high density of bacteria to be diagnosed in 5 ml of physiological solution to give the turbidity of 5%.
2. Inject this bacterial suspension in each tube of the twenty tubes and the transition them without air bubbles using sterile Pasteur.
3. Provide anaerobic conditions in the tubes (urease and arginine hydrolase) by adding oil paraffin to the hole.
4. Put the tape in its own box with a little water in the bottom of the can to prevent dehydration during incubation at 35°C at 24 hours.
5. The required detectors have been added to the examination tubes listed below:

    a. VP (Acetyl-Methyl-Carbinol)
    b. NTI (Reduction of nitrate to nitrite)

For laboratory tests, solid samples were mixed with 25 g of meat and 250 ml of the buffer solution at pH 7.2. Samples were homogenized using a blender at 2 min. Then the dilution of the mixture was done for the counting bacteria. Then 0.1 ml of dilution solution was added to the Baird-Parker Media and spread by an L-shaped glass rod. Then obtained bacterial colonies were perpetrated. The fixation of bacteria for staining, gram stain, and biochemical tests are performed. The analysis of plasma coagulation was performed using the rabbit blood plasma. The catalytic analysis was conducted using hydrogen peroxide of 3%, oxidase analysis was conducted using N, N, N, tetramethyl p-phenylenediamine hydrochloride, and blood analysis was conducted on 5% (H / H) from human blood. These tests were done using several APIs Staph by the French company BioMerieux after diluting of the colonies in 5 ml of the physiological solution supplied by the company.

Then 250 ml of physiological water were added to 25 g of meat and then 5n HCL added to the

mixture and leave it about 15-30 minutes at 18-25°C followed by the centrifuge for 15 minutes and speed of 3000-5000 and then the solution was filtered using a syringe. 1N NaOH solution was added to the isolated sample (pH8). Each 500 µL of VIDAS was transferred for determining the concentration and type of the toxin.

Table 1. Biochemical properties

No	Tests	Characteristic
1	Gram stain	Positive
2	Figures microscope	Cluster cocci
3	Catalase	Positive
4	Oxidase	Negative
5	Coagulase	Positive
6	Nitrate reduces	Positive
7	Fermentation	Positive

3. RESULTS AND DISCUSSION

Chromogenic medias of *S. aureus* were black and pink colonies (Fig. 1).

All samples were divided into two groups, as shown in Table 2. The first group was contained of 60 samples, which was contaminated by *S. aureus*. The second group was contained of 40 samples, which was without *S. aureus*.

Table 2. Chicken breasts with bacterial growth

Groups	Samples	Bacteria type
1	60	Growth of *S. aureus*
2	40	Non-growth of *S. aureus*
Total	100	---

The first group was divided depending on toxin, as shown in Table 3.

Sixty samples were distributed to 7 groups, each group was contained bacterial concentrations in CFU ml^{-1}, as shown in Table 3. All toxins were less than 0.13 ng g^{-1}, which were negative, but other groups (3-7) of toxin of 10^4–10^8 CFU ml^{-1} were positive toxin. The results in the A-type have a high concentration in all samples. The concentrations were ranged between 0.25 ng g^{-1} and 2.00 ng g^{-1}. Other results of toxins (10^7 – 10^8 CFU ml^{-1}) were showed the toxin of B-type between 1.00 ng g^{-1} and 1.00 ng g^{-1} in groups 6 and 7, respectively.

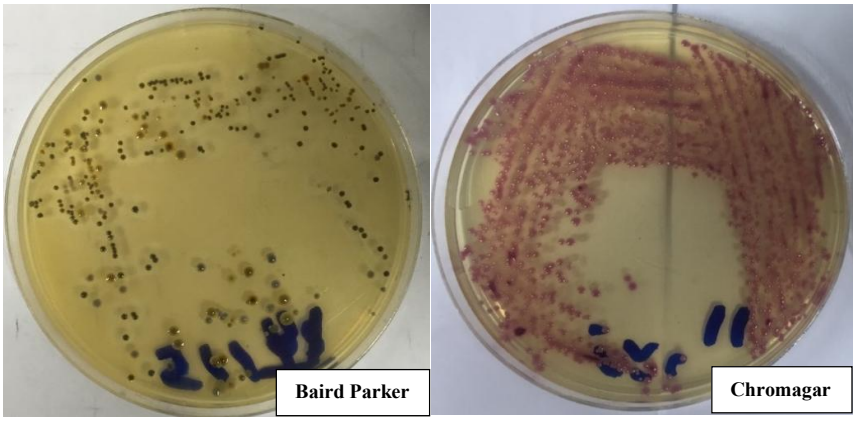

Fig. 1. *S. aureus* was isolated on both chromagar and Baird parker medias

Table 3. Toxin concentration and colony (CFU ml^{-1})

Groups	Samples	Samples%	Colony	Toxins type with concentration (ng g^{-1})				
-	-		-	A	B	C	D	E
1	9	15	40×10^2	0.00	0.00	0.00	0.00	0.00
2	5	0.8	35×10^3	0.00	0.00	0.00	0.00	0.00
3	10	16.6	29×10^4	0.25	0.00	0.00	0.00	0.00
4	10	16.6	22×10^5	1.00	0.00	0.00	0.00	0.00
5	15	25	18×10^6	1.00	0.00	0.00	0.00	0.00
6	8	13.3	14×10^7	2.00	1.00	0.00	0.00	0.00
7	3	5	12×10^8	2.00	1.00	0.25	0.00	0.00
Total	**60**			**Cut off**			**0.13 ng g^{-1}**	

Toxin C in the group 7 at 10^8 CFU ml^{-1} of 0.25 ng g^{-1} was found. The bacterial concentrations were found to be about 10^6 CFU ml^{-1} in some literatures review. Whereas, in this study, the toxin was found about between 10^4 to 10^5 CFU ml^{-1}. This study is a new measurement of the toxic using VIDAS technique. Chicken products were isolated with positive *S. aureus* of 37.5%, whereas meat was found to be 60% [16]. The toxicity was found to be 0.25, which was positive for the toxic A. When the bacteria concentration increases, the poison concentration increases as new toxicity types, which were shown using the Enzyme Linked Fluorescence Assay based on VIDAS system. VIDAS SET 11 was detected the toxin of enterotoxin of *S. aureus*. The SET II was contained 4 sensitive levels including 0.0 ng g^{-1}, 0.25 ng g^{-1}, 1.00 ng g^{-1}, 2.00 ng g^{-1}, and 0.13 ng g^{-1} (cut off). Then the result > 0.13 ng g^{-1} was positive, whereas the result < 0.13 ng g^{-1} was negative.

4. CONCLUSIONS

The toxin was produced at 10^6 CFU ml^{-1} in all other scientific researches, but in this study, it was found that the toxin was produced at 10^4 and 10^5 CFU ml^{-1}. It was concluded that the contamination is due to poor handling of the samples. These results make this research as a distinguished research in the worldwide.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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